**Histone-associated thrombocytopenia in critically ill patients**

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**Introduction**

Thrombocytopenia is observed in approximately 30% to 40% of intensive care unit (ICU) patients and associated with poor outcomes. Histones induce profound thrombocytopenia in mice,1,2 and are associated with organ injury when released following extensive cell damage in critically ill patients. 3,4 We explored the association between circulating histones and thrombocytopenia in ICU patients.

**Methods**

A case-control study was performed including general ICU patients admitted to the Royal Liverpool University Hospital between June 2013 and January 2014 with approval from North West Centre of Research Ethics Committee, UK. Written informed consent was obtained. Thrombocytopenia was defined as a platelet count less than 150x103/µl, a 25% or greater decrease in platelet count, or both5 within the first 96 hours of ICU admission (study duration). Patients with known prior cause(s) of thrombocytopenia were excluded. The control group was ICU patients without thrombocytopenia during the same study duration, matched to thrombocytopenic patients for age, sex, APACHE II scores and admission diagnoses. Plasma histones were measured, as described previously,3,4 and daily levels (median [first, third quartiles]) were compared between thrombocytopenic patients and non-thrombocytopenic controls. As histones at approximately 30µg/ml bind platelets and cause platelet aggregation, resulting in profound thrombocytopenia in mice,1,2 this level was used to stratify thrombocytopenic patients (high admission histones ≥30µg/ml; low, <30µg/ml). Platelet counts and percentage decrease in platelet counts at 24 and 48 hours post-admission were compared between these 2 thrombocytopenic groups. In addition, daily histone levels were compared between patients with mild (platelets 100-149x103/µl), moderate (platelets 50-99x103/µl) and severe (platelets <50x103/µl) thrombocytopenia. Mann-Whitney U test was used for comparisons. Receiver Operating Characteristic (ROC) curve assessed the performance of admission histone levels in predicting moderate-severe thrombocytopenia during the study. Chi-squared test was used for categorical groups (sex, presence/absence of certain diagnosis, presence/absence of circulating histones). Statistical tests were performed on SPSS software (version 22). *P*<.05 (2-sided) defined statistical significance.

**Results**

56 thrombocytopenic patients and 56 non-thrombocytopenic controls were studied. Circulating histones were detectable in 51 (91%) thrombocytopenic patients compared with 31 (55%) controls (*P*<.001). Daily histone levels were significantly higher in thrombocytopenic patients compared with controls throughout the study (Table).

Thrombocytopenic patients with high admission histones (n=32) had significantly lower platelet counts at 24 and 48 hours post-admission compared with thrombocytopenic patients with low admission histones (n=24) (Figure 1A). Thrombocytopenic patients with high admission histones had significantly greater percentage decreases in platelet counts at 24 and 48 hours post-admission, compared with thrombocytopenic patients with low admission histones (Figure 1B).

Histone levels on admission and 24 hours post-admission were significantly higher in patients developing severe or moderate thrombocytopenia compared with mild thrombocytopenia (Table). Admission histone levels were associated with development of moderate-severe thrombocytopenia with an area under ROC curve of 0.893 (95%CI 0.843-0.944, *P*<.001). At 30µg/ml histone concentration, the sensitivity and specificity were 76% and 91%, respectively (positive and negative predictive values=79.4% and 89.2%; positive and negative likelihood ratios=8.5 and 0.2, respectively).

**Discussion**

In this study, histones circulated in the majority of thrombocytopenic patients and were 2.5-5.5 fold higher than in non-thrombocytopenic controls. There was a significant association between high admission histones and subsequent decline in platelet counts among thrombocytopenic patients. High admission histones were associated with moderate-severe thrombocytopenia and development of clinically important thrombocytopenia with high area under ROC curve.

The limitations of this study include a relatively small number of patients from a single center and the difficulty in establishing a cause-effect relationship between circulating histones and thrombocytopenia without interventional studies.

Circulating histones are potential markers of disease severity,3,4,6 and the association with thrombocytopenia may reflect this. Nevertheless, the novel associations reported in this study extend previous reports demonstrating profound thrombocytopenia following histone infusion into mice1,2 and suggest that, if confirmed, circulating histones may be valuable in predicting or monitoring thrombocytopenia in critically ill patients.

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**Figure legends**

**Figure 1. Circulating histones are associated with thrombocytopenia in critically ill patients.**

A, Platelet counts at 24 and 48 hours post-ICU admission in thrombocytopenic patients with low (<30µg/ml, n=24) and high (≥30µg/ml, n=32) admission circulating histone levels. Horizontal bars represent median levels. *P* values are calculated by Mann-Whitney U test. B, Percentage decrease in platelet counts at 24 and 48 hours post-ICU admission in thrombocytopenic patients with low (<30µg/ml, n=24) and high (≥30µg/ml, n=32) admission histone levels. Percentage decrease in platelet counts is calculated in reference to platelet count on admission. Horizontal bars represent median levels. *P* values are calculated by Mann-Whitney U test.

**Tables**

**Table 1. Patients’ characteristics**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Thrombocytopenia  ICU patients | | | | | ICU  controls |  |
|  | Mild a | Moderate a | Severe a | Total | *P* b | Total | *P* c |
| n | 18 | 23 | 15 | 56 |  | 56 |  |
| Age (years) d | 65[48,71] | 62[49,76] | 63[51,75] | 64[49,75] | .74 / .68 | 65[49, 73] | .97 |
| Male (n) [%] | 13/18[72%] | 14/23[61%] | 9/15[60%] | 36/56[64%] | .52 / .71 | 28/56[50%] | .18 |
| APACHE II score d | 17[15,24] | 21[14,28] | 25[18,31] | 21[16,27] | .66 / .06 | 20[12,24] | .21 |
| Platelet count (x103/µl) d  Admission  24 hours post-admission  48 hours post-admission  72 hours post-admission | 166[139,224]  140[130,152]  125[117,136]  118[107,129] | 128[94,148]  96[84,120]  91[73,107]  88[77,100] | 64[43,89]  47[40,80]  47[31,73]  42[27,85] | 123[83,166]  102[80,140]  96[68,122]  98[71,116] | <.001  <.001  <.001  <.001 | 219[177,301]  222[183,289]  215[182,299]  216[182,261] | <.001  <.001  <.001  <.001 |
| Plasma histone levels (µg/ml) d  Admission  24 hours post-admission  48 hours post-admission  72 hours post-admission | 4[2,32]  5[2,15]  15[0,37]  22[11,43] | 40[15,74]  35[11,60]  22[8,47]  17[9,37] | 39[31,109]  48[33,96]  26[25,42]  24[17,35] | 33[5,55]  29[6,57]  25[9,42]  22[12,43] | .002 / .001  .004 /<.001  .29 / .07  .81 / .69 | 6[1,11]  9[1,17]  9[1,17]  9[2,13] | <.001  .001  .001  <.001 |
| Admission diagnosis, n [%] e  Sepsis  Trauma  Cardiovascular  Respiratory  GIT f  Renal  CNS g | 5 [8.9%]  3 [5.3%]  4 [7.1%]  2 [3.5%]  3 [5.3%]  1 [1.8%]  0 [0%] | 4 [7.1%]  5 [8.9%]  4 [7.1%]  3 [5.3%]  4 [7.1%]  2 [3.5%]  1[1.8%] | 4 [7.1%]  1 [1.8%]  2 [3.5%]  3 [5.3%]  3 [5.2%]  2 [3.5%]  0 [0%] | 13 [23.1%]  9 [16%]  10 [17.7%]  8 [14.1%]  10 [17.7%]  5 [8.9%]  1[1.8%] | .47 / .99  .99 / .60  .50 / .38  .99 / .63  .99 / .99  .99 / .58  .99 / >.99 | 10 [17.7%]  11 [19.6%]  8 [14.1%]  12 [21.4%]  7 [12.5%]  3 [5.3%]  5 [8.9%] | .63  .80  .79  .46  .59  .71  .20 |

SI conversion factor: to convert platelet counts to x109/L, multiply values by 1.

a Mild thrombocytopenia (platelets 100-149x103/µl), moderate thrombocytopenia (platelets 50-99x103/µl), severe thrombocytopenia (platelets <50x103/µl). b *P* values are presented for moderate thrombocytopenia vs mild thrombocytopenia / severe thrombocytopenia vs mild thrombocytopenia (one *P* value is provided if the two *P* values are the same).c *P* values are presented for thrombocytopenia ICU group (total) vs ICU control group with no thrombocytopenia (total). d Data are presented as median [1st quartile, 3rd quartile] and statistical significance determined by Mann-Whitney U test. e Percentages are calculated as [number of cases in a particular diagnosis/56 (total number of cases) x100].f Gastro-intestinal. g Central nervous system.